

Claims

1. A method of producing a biologically active oligomeric form of α -lactalbumin, which method comprises contacting α -lactalbumin, at least some of which is in the molten globule-like state, with a conversion reagent which results in the production of said biologically active oligomeric form.
2. A method according to claim 1 wherein α -lactalbumin, at least some of which is in the molten globule-like state, is contacted with said conversion reagent under conditions which allow ion exchange to take place.
3. A method according to claim 1 wherein α -lactalbumin in the molten globule-like state is applied to an ion exchange column, which contains the conversion reagent.
4. A method according to claim 2 wherein the ion exchange column is an anion exchange column.
5. A method according to claim 2 or claim 3 wherein the ion exchange column has been eluted with the conversion reagent.
6. A method according to any one of claims 1 to 5 wherein a substantial portion of the α -lactalbumin is in the molten globule-like state.
7. A method according to claim 6 wherein the α -lactalbumin is subjected to a pre-treatment step in order to maximise the amount of molten globule-like material present.

8. A method according to claim 7 wherein in the pre-treatment step, the α -lactalbumin is contacted with a calcium chelating agent.
- 5 9. A method according to claim 8 wherein the calcium chelating agent is ethylene diamine tetraacetic acid.
- 10 10. A method according to claim 7 wherein the pre-treatment step comprises exposure to a low pH.
11. A method according to claim 10 wherein the low pH is created by addition of hydrochloric acid, so as to reduce the pH to the order of 2.
- 15 12. A method according to claim 8 wherein the pre-treatment step comprises heating the α -lactalbumin to a temperature of from 25-120°C.
- 20 13. A method according to claim 12 wherein the temperature is from 70°C-120°C.
- 25 14. A method according to any one of claims 1 to 6 wherein α -lactalbumin is applied to an ion exchange column together with a reagent which will induce it to form the molten globule-like state.
- 30 15. A method according to claim 14 wherein the molten globule inducing reagent is a calcium chelating agent which is present in an elution buffer.
16. A method according to claim 15 wherein the calcium chelating agent is EDTA.
- 35 17. A method according to any one of claims 1 to 16 wherein the conversion reagent comprises a fatty acid or lipid which is able to act as a conversion reagent.

18. A method according to claim 17 wherein the fatty acid or lipid is a component of casein.
- 5 19. A method according to claim 17 or claim 18 wherein the fatty acid is oleic acid.
20. A method according to any one of the preceding claims wherein the α -lactalbumin is a mutated form of the native
10 protein in which calcium binding sites are modified.
21. A method according to claim 20 wherein at least some cysteine residues of the α -lactalbumin are mutated.
- 15 22. A method for producing an oligomeric form of α -lactalbumin which comprises exposing a source of α -lactalbumin to an ion exchange medium which has been pre-treated with a casein containing fraction of milk, or an active component thereof and recovering α -lactalbumin in an
20 oligomeric form therefrom.
23. A method according to claim 22 wherein the active component of casein is oleic acid.
- 25 24. A method according to claim 23 wherein the oleic acid is in substantially pure form.
25. A method according to claim 22 wherein the ion exchange medium has been treated with a casein containing
30 fraction derived from human milk.
26. A method according to claim 25 wherein the ion exchange medium has been treated with a casein containing milk fraction which has been previously frozen or is
35 derived from frozen milk.

27. A method according to claim 25 or claim 26 wherein the casein used in the pre-treatment of the ion exchange medium has been subjected to hydrolysis.
- 5 28. A method according to any one of claims 22 to 27 wherein a substantial portion of the α -lactalbumin applied to the ion exchange medium in the molten globule-like state.
- 10 29. A method according to claim 28 wherein the α -lactalbumin is formed into the molten globule-like state by contacting it with a calcium chelating agent.
- 15 30. A method according to claim 29 wherein the calcium chelating agent is ethylene diamine tetraacetic acid.
31. A method according to claim 29 or claim 30 wherein the calcium chelating agent is contacted with the α -lactalbumin prior to contact with the ion exchange medium.
- 20 32. A method according to claim 30 or claim 31 wherein the calcium chelating agent is added to an elution buffer which is then used to effect the contact between the α -lactalbumin and the ion exchange medium.
- 25 33. A method according to claim 26 wherein the α -lactalbumin is subjected to pre-treatment step involving exposure to a low pH.
- 30 34. A method according to claim 26 wherein the α -lactalbumin is subjected to a pre-treatment in which it is heated to an elevated temperature.
- 35 35. A method according to any one of claims 28 to 34 wherein the ion exchange medium is arranged in a column.

36. A method according to any one of claims 28 to 35 wherein the ion exchange medium comprises DEAE Trisacryl.

37. A method according to any one of claims 28 to 36 which
5 comprises passing a casein containing milk fraction or one or more active components thereof in an ion exchange buffer down an ion exchange column, washing the column with ion exchange buffer, and then passing a source of α -lactalbumin dissolved in the ion exchange buffer down the ion exchange
10 column in the presence of a salt concentration gradient.

38. A method according to claim 37 wherein the ion exchange buffer is Tris-HCl.

39. A method according to claim 37 or claim 38 wherein the
15 salt concentration gradient is produced using an ion exchange buffer in which sodium chloride is dissolved.

40. A method according to claim 39 wherein the column is
20 washed by elution of ion exchange buffer twice.

41. A method according to any one of the preceding claims wherein the said source of α -lactalbumin comprises monomeric bovine α -lactalbumin.

25 42. A method according to any one of claims 1 to 40 wherein the said source of α -lactalbumin comprises monomeric human α -lactalbumin.

30 43. An ion exchange medium for use in the method of any one of the preceding claims, said medium having been treated with a casein containing milk fraction or an active component thereof.

44. An ion exchange medium according to claim 22 wherein the medium has been treated with an active component of casein containing milk fraction comprising oleic acid.

5 45. An ion exchange column which comprises ion exchange medium according to claim 43 or claim 44.

46. An oligomeric form of α -lactalbumin obtained by a method according to any one of claims 1 to 42.

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AMENDED SHEET